TIRC Grant #252

Progress Report #1

O. J. Pollak, M.D., Ph.D.

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CONFIDENTIAL

DOVER MEDICAL RESEARCH CENTER, INC.
Office: 9 Kings Highway, E., P.O. Box 228
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October 3, 1960

Robert C. Hockett
Assoc. Scientific Director
TOBACCO INDUSTRY RESEARCH COMMITTEE
150 East 42nd Street
New York 17, N.Y.

Dear Mr. Hockett:

Time is surely flying and six months have passed since the project under the grant of the Tobacco Industry Research Committee was activated.

Enclosed find a brief report which will bring you up to date. It seems that the beginning is the hardest and it is surely hoped that in the future progress will be speedier. I personally feel that our observations are very interesting and certainly warrant continuation. They also will warrant expansion utilizing material from other species...especially human material. Projecting into the future I would say that it would take us another six months to study blood vessels and heart muscle of rabbits. Many changes had to be made in the course of studies. The amount of cells does not lend itself to metabolic studies and, therefore, we have started to utilize homogenates of vascular tissue for Warburg studies. This work has, unfortunately, not yet progressed very far. We are still trying to get the base lines; that is, differences between the oxygen consumption of tissue from normal rabbits, rabbits with induced blood chemical changes but without visible vascular changes and, finally, tissues from rabbits with hypercholesteremia and hyperlipemia and, also, aortic atherosclerosis. Only after we get the base lines can we start adding nicotine in varying concentrations to these 3 types of material.

Certainly, the studies should not be confined to nicotine but will have to embrace other ingredients of tobacco as well as tobacco smoke. With this in mind, I would somehow like to imply that another application for continued support of these studies will be forthcoming. Naturally, we don't want to let the grant expire before we apply for continuation and I am awaiting your personal advice as to the proper procedure for the future.

Sincerely yours,

/s/ O. J. Pollak, M.D., Ph.D.
Executive Director

OJP:pkg

Effect of Tobacco Derivatives on Arterial and Myocardial Tissue Cultures

Considerable time had been spent establishing "controls", i.e.; cultures of material of healthy rabbits and from rabbits rendered hypercholesteremic. At first, spindle cells of fibroblastic character were cultured, only. It became necessary to find optimum conditions for growth. (A report on these studies has been submitted for publication.) Modifications of technics led to successful cultures of intimal endothelium cells from normal aortae and from experimental intimal plaques. (A report on these studies is being prepared for publication.)

Nicotin has been added tc culture medium in three concentrations, 1:40,000, 1:10,000 and 1:2,500. The amount of nicotin solution added to medium was 0.04 ml. per 100 ml. The two weaker solutions (1:40,000 and 1:10,000) had no effect on the number of positive cultures. In all three concentrations nicotin delayed the cell growth. In cultures from healthy aortic tissues, the number of cells was reduced by almost one-half in medium with dilute nicotin solutions (1:40,000 and 1:10,000) and by two-thirds in concentrate solution (1:2,500). Cells from atheromatous plaques were more sensitive: the number of cells was reduced by about one-third in medium with 1:40,000 nicotin solution, by almost one-half with 1:10,000 solution and by about 90 per cent in medium with 1:2,500 solution of nicotin, at a given date. Morphologic changes of fibroblasts were seen in medium with 1:2,500 solution but not with more diluted nicotin. Endothelium cells were more sensitive: they became severly vacuolized where nicotin solutions of 1:2,500 or 1:10,000 had been added to the media. Degranulation of the cells and nuclear pyknosis occurred even where 1:40,000 nicotin solution had been added. Phagocytic ability of cells was also affected. The life span of endothelium cells was considerably shortened by addition of 1:2,500 nicotin solution to the medium.

The number of animals studied is, as yet, small. The studies have to be expanded to add validity to our observations. Studies of myocardial cells are not conclusive, so far.

Amino acid assays of culture medium before planting and after cell growth have been started. The number of assays is too small to allow evaluation. Lack of "pure" cultures of endothelium cells presents a problem.

Until now, nicotin has been added to medium before planting. Addition of nicotin solutions to positive cell cultures will be started, shortly.

Since the total number of cells grown is hardly sufficient for Warburg studies and since it is hardly possible to obtain pure lines of endothelium cells it will be necessary to modify part of the research project. We plan to prepare homogenates from aorta and myocardium of healthy and of hypercholesteremic rabbits, subject these to metabolic studies and then investigate the influence of nicotin additives.